Polylysine modified hydrogels: brain mimetic materials for neural tissue engineering

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Introduction

Every year over a million individuals suffer from loss of neuronal functions as a result of neurodegenerative disorders [1]. Unfortunately, the central nervous system (CNS) neurons do not possess regeneration potential following injury [2]. Several biomaterials (both natural and synthetic) have been developed that find extensive applications as tissue engineered scaffolds or as neuro-prosthetic components [3]. Natural biomaterials developed for neural tissue engineering mimic the native tissue environment; however their extremely complex structure leaves less scope for further manipulation. On the other hand, properties of synthetic materials (i.e., chemical and mechanical) can be fine tuned for a particular application very easily. This makes them attractive candidates for neural tissue engineering applications [2]. However, most of the synthetic polymers do not support neural cell adhesion, an important event in tissue regeneration. Releasing this major setback, it is vital to enhance the cell adhesive capability of synthetic neural biomaterials. To address this concern, these polymers are modified with adhesion molecules [4] (i.e., proteins/peptides that modulate neuronal adhesion). Incorporation of these molecules would improve integration of synthetic materials with the body. In addition to neural tissue engineering, these polymeric materials could be used as neural prosthesis coatings to help improve integration of the device [5] with the tissue and promote chronic neural interfaces. To achieve these goals, we developed synthetic hydrogels, cross linked, hydrophilic polymeric materials, that closely mimic the native brain tissue. In particular, we investigated adhesion molecule modified-poly

(ethylene glycol) (PEG) based hydrogel materials as potential coatings for prosthetic devices and as neural tissue mimetics. We chose polylysine (PL) as the adhesion molecule because of its ability to promote neuronal adhesion in traditional 2D cultures [6]. PEG was chosen because of its non-immunogenic properties and biocompatibility [7]. PL-modified PEG-hydrogel materials were developed, characterized and investigated for their ability to promote neural cell adhesion using a model PC12 cell line. Adhesion molecule-modified hydrogels offer great promise as neural tissue mimetic materials as well as prosthetic device coatings for stimulation and/or recording electrodes.

Experimental Details

PEG, in its diacrylate form can form hydrogels under the influence of UV illumination in the presence of an initiator. Modification of the polymeric hydrogel with adhesion molecule was performed by incorporating polylysine (into backbone of poly (ethylene glycol)-diacrylate (PEG-DA) hydrogels using standard bioconjugation techniques [8]. PEG-DA hydrogels were prepared using standard protocols [9]. PL was conjugated to Acryl-PEG-N- hydroxysuccinimide (Acryl-PEG-NHS) using the standard NHS chemistry. Briefly, PL (Mw 30,000-70,000) dissolved in 50mM sodium bicarbonate solution and Acryl-PEG-NHS (Mw 3400) dissolved in DMSO, were mixed in a 1:2 molar ratio. They were then allowed to react for about 2 hrs in an ice bath. Resulting samples were subjected to dialysis and further reconcentrated to their original volumes using centrifugal concentrators. The resulting Acryl-PEG-PL was then incorporated into PEG-DA *base* hydrogels using UV photopolymerisation (Figure 1). For characterizing PL conjugation, FITC poly-L-lysine (PLL) was used with poly-D-lysine (PDL) in 1:10 weight ratio, whereas PDL alone was used for all other experiments. Polylysine conjugation was monitored by observing the diffusion of FITC-polylysine over a period of one week against a negative control

(no polylysine) and a sham (unconjugated polylysine) using a fluorescent plate reader. Extent of conjugation was evaluated using standard PL concentrations. Data was analyzed using ANOVA and Tukey Kramer's HSD ($\alpha = 0.05$) JMP statistical software (Version 7).

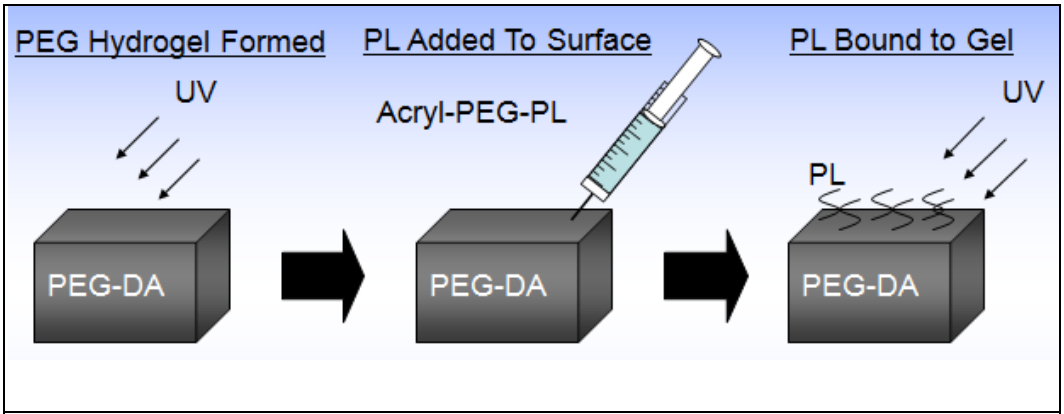

Figure 1: Conjugation of PL to PEG hydrogels (Schematic of the procedure).

In order to evaluate cell response on PL-modified materials, we chose PC12 cell line, a model cell line for studying neural behavior [10-12]. PC12 cell response (i.e., neural adhesion) was then assessed using polylysine conjugated materials against suitable controls. Briefly, PDL conjugated PEG hydrogels were prepared as described previously. Prior to gelation, all solutions were sterile filtered using a syringe filter (pore size 0.22µm). Hydrogels were then washed with sterile phosphate buffer saline (PBS) for one week and subjected to UV illumination overnight (for sterilization). PC12 cells (2×10^4 cells/cm^2) were then seeded on all surfaces (PL-conjugated samples, unconjugated samples, samples containing no PL). After 24h, all surfaces were washed with sterile PBS and then cells were stained using Live-dead staining. Following 45 min incubation, hydrogels were again washed with sterile Dulbecco's PBS (D-PBS) to remove excess dye and their fluorescence was observed. Fluorescence intensity (for live cells, stained green) was used as an indirect measure to quantify cell adhesion to modified hydrogel surfaces. A schematic of PL-modified PEG hydrogels is shown in Figure 2.

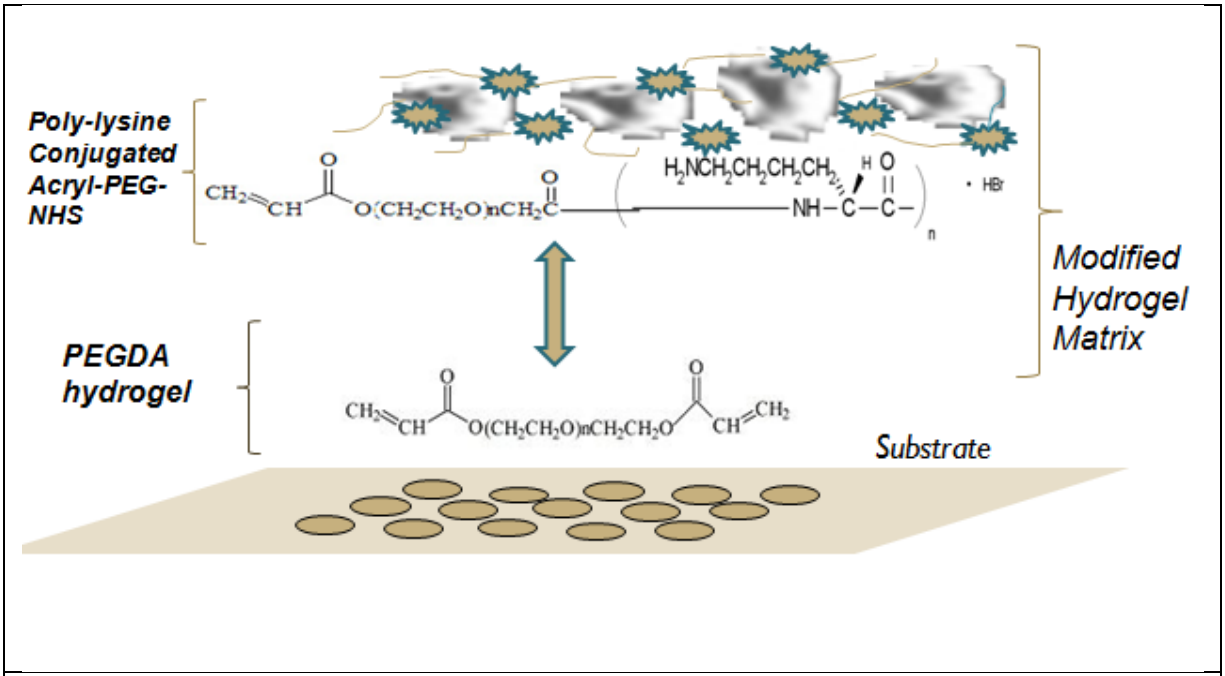

Figure 2: PL-modified PEG-hydrogels (Schematic)

Results and Discussion

The amount of PL incorporation into PEG hydrogels was determined using fluorescence spectroscopy. As shown in figure 3, the amount of covalently bound PL at day 7 was ~0.095 µg/µl and the amount of entrapped PL at day 7 was ~ 0.27µg/µl, both of which were significantly different from the negative control (no PL) ($p < 0.0001$*) [Note that the conjugated PL in Figure 3 consists of conjugated as well as entrapped PL]. In both sample and sham (unconjugated PL), PL was incorporated into the PEG matrix, either as a bound or unbound (entrapped) molecule. In both these cases, limited diffusion of PL from the hydrogel matrix was observed, thereby indicating formation of a stable adhesion molecule-modified hydrogel matrix.

Adhesion molecule (PL)-modified hydrogels promote PC12 cell adhesion with PL-conjugated hydrogel as well as PL entrapped hydrogel having significantly different fluorescent intensities as compared to negative control [Fluorescence intensity was used as a measure of cell number

since it is proportional to cell number]. However, this was observed only at lower PL concentration regimes. At higher PL concentration, excessive cell death was evident (Figure 4). This is not surprising, since higher concentrations of PL have been previously reported to be cytotoxic resulting in cell lysis[13].

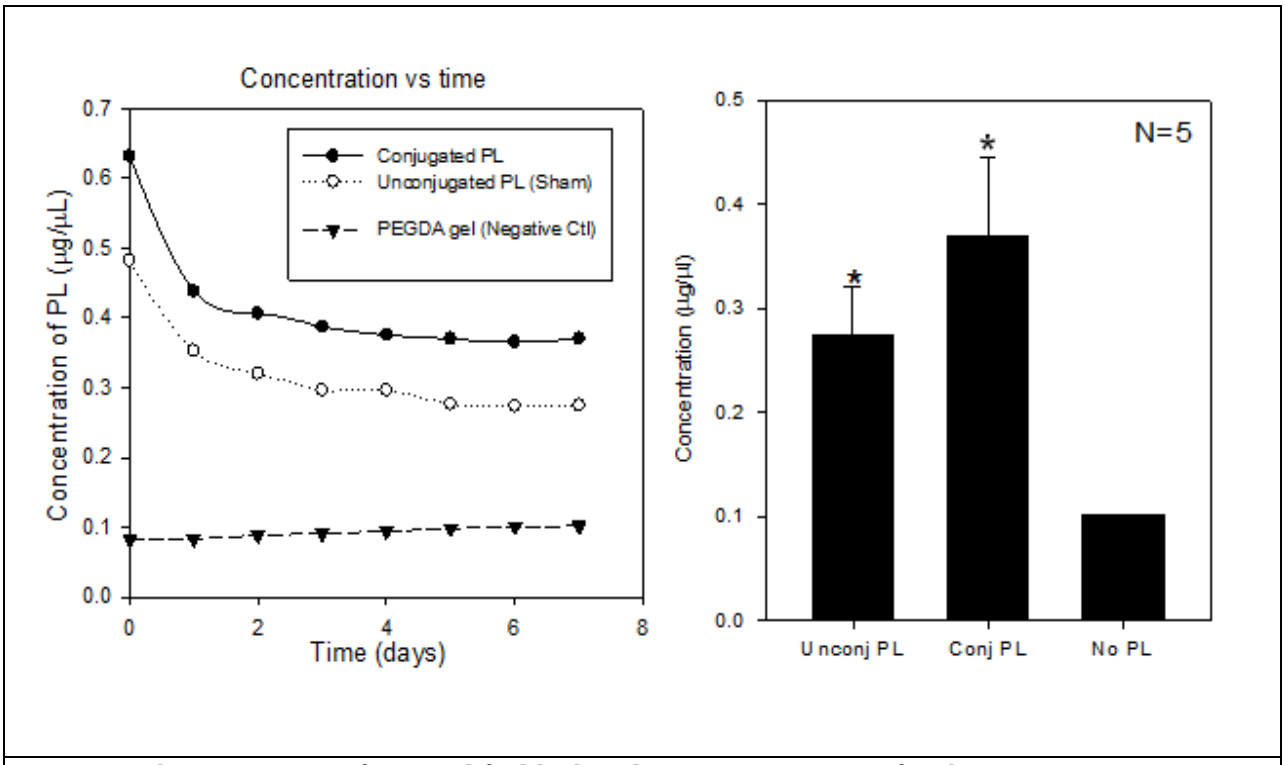

Figure 3: Characterization of PL-modified hydrogels using FITC-PL. Left, Change in concentration vs. time (elution of unconjugated PL).Right, Concentration of conjugated and unconjugated PL at the end of 1 week. *indicates significant difference from negative ctl (No PL) ($p < 0.0001$*)

Both sample and sham hydrogels were equally able to promote adhesion of PC 12 cells in the lower concentration regime (Figure 4). This could be further explained by the mechanism by which PL promotes cell adhesion. Positively charged PL attracts the negatively charged cell membrane thereby aiding in cell adhesion. This mechanism is referred to as 'non-receptor mediated cell binding'[4]. This is in contrast to other adhesion molecules (e.g., laminin, collagen) which promote cell binding via the receptor mediated mechanisms. With these molecules, chemical conjugation may be necessary for the cells to generate a certain amount of

traction force to support adhesion. Further, adhesion in the case of these molecules is facilitated by certain specific binding sites (e.g., RGD for collagen[4]). If these molecules are entrapped in the matrix, steric hindrance, in some cases, may hinder the process of adhesion. Since PL promotes adhesion through charge based mechanisms, our results suggest that conjugation of PL to the hydrogel matrix may not be always necessary.

Cell response to these materials was assessed using only one cell type. It would be interesting to study cell response of other relevant cells (e.g., astrocytes) in the CNS to these materials as well.

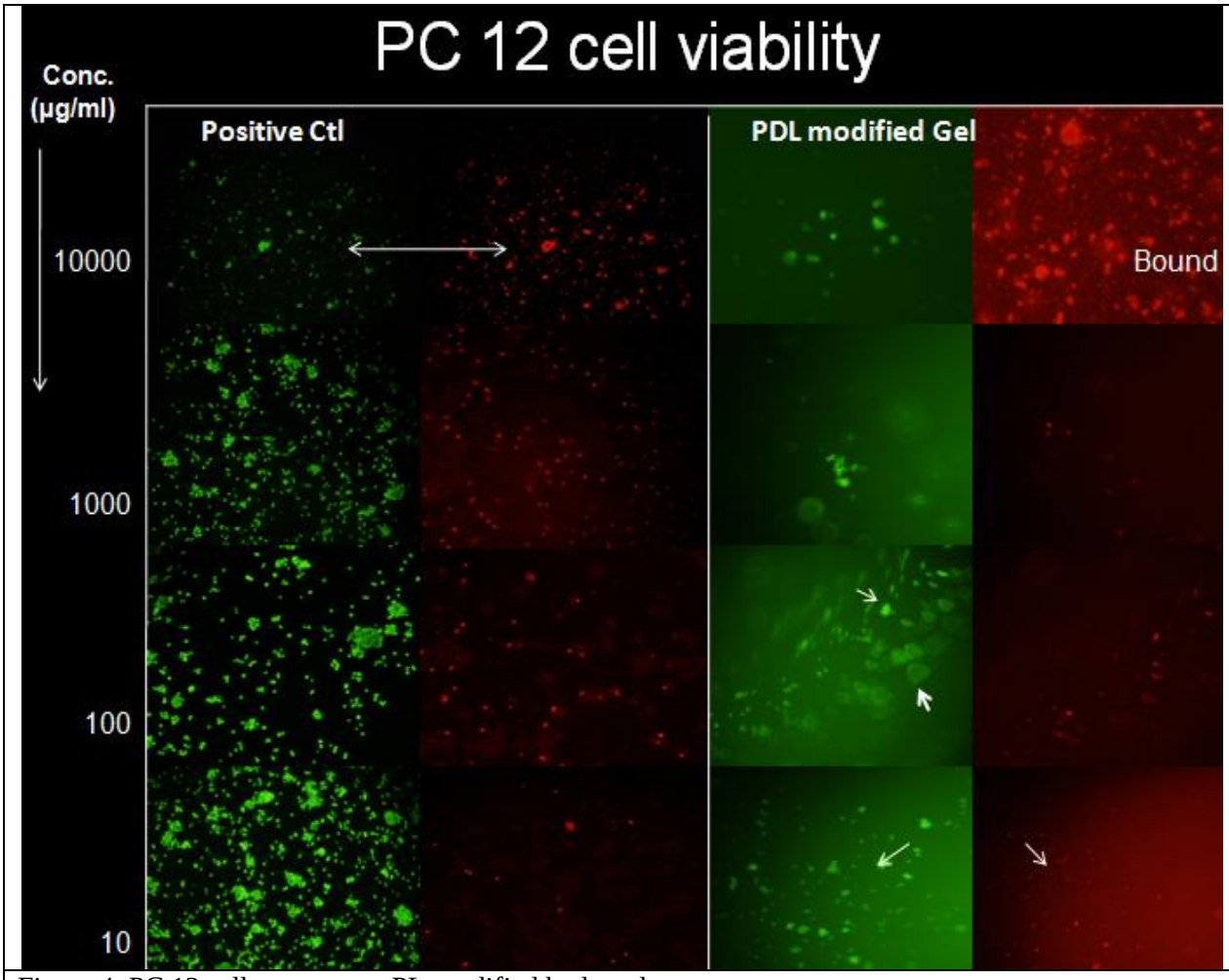

Figure 4: PC 12 cell response to PL-modified hydrogels.

PL-modified hydrogel materials represent one class of biomaterials that could be used as surface coatings for neural stimulation or recording electrodes to improve electrode-tissue biocompatibility and enhance device performance. However, our observations also suggest PEG-DA hydrogels does not adhere to electrode surfaces. Therefore, this technique would be utilized in conjugation with other PEG-based copolymers synthesized in our laboratory (e.g., poly (ethylene glycol) poly (lactic acid) (PEG-PLA), poly (ethylene glycol) poly (caprolactone), (PEG-PCL)) that form hydrogels on exposure to UV illumination. In addition, we are also working on creating patterned hydrogel surfaces to evaluate specific cell responses (Figure 5).

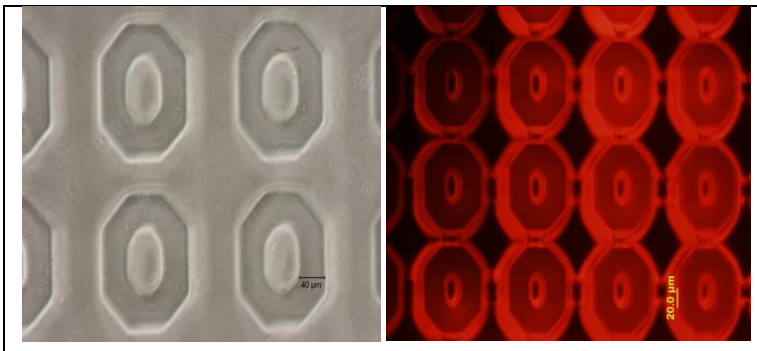

Figure 5: Patterned PEG hydrogels. Courtesy of Dr. Wen

Conclusions

Adhesion molecule (PL)-modified hydrogels were developed that could be employed as neural tissue mimetic materials or as coatings to enhance the interface between electrode and the host tissue. PL was conjugated to PEG hydrogels and its attachment was confirmed by fluorescence spectroscopy. PL modified hydrogel materials support neuronal cell adhesion thereby encouraging tissue regeneration. Future work would involve investigating the effect of incorporating multiple cues (e.g., simultaneous application of growth factors and adhesion molecules, patterned biomimetic materials) within the hydrogel coating with an aim to apply these materials to enhance the nerve tissue-electrode interface.

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